



UNITED STATES
DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE
ECOLOGICAL SERVICES
3616 W. Thomas, Suite 6
Phoenix, Arizona 85019

2-21-90-F-275

December 14, 1990

MEMORANDUM

TO: District Manager, Arizona Strip District, Bureau of Land Management,
St. George, Utah

FROM: Field Supervisor

SUBJECT: Mohave County Road Right-of-Way - Biological Opinion

This Biological Opinion responds to your memorandum dated September 5, 1990, which requested formal consultation with the Fish and Wildlife Service (FWS) pursuant to Section 7 of the Endangered Species Act of 1973, as amended (Act). The FWS received your request on September 10, 1990. The action under consultation involves issuance of a right-of-way (A-24577) on Bureau of Land Management (BLM) lands for construction of a county road and its impacts on the desert tortoise (Gopherus agassizii), a Federally listed threatened species.

This Biological Opinion was prepared using the best project description and on-site biological information available at the time of consultation, including: Section 7 Evaluation (September 4, 1990), Environmental Assessment (August 14, 1990), maps showing compensation (November 21, 1990), an on-site inspection on September 6, 1990, discussions with your staff, and information contained in our files. The Desert Tortoise Habitat Management on the Public Lands: A Rangewide Plan (Rangewide Plan) was used as guidance for determining management objectives for tortoise habitat, especially Rangewide Plan Objective 8 (BLM 1988).

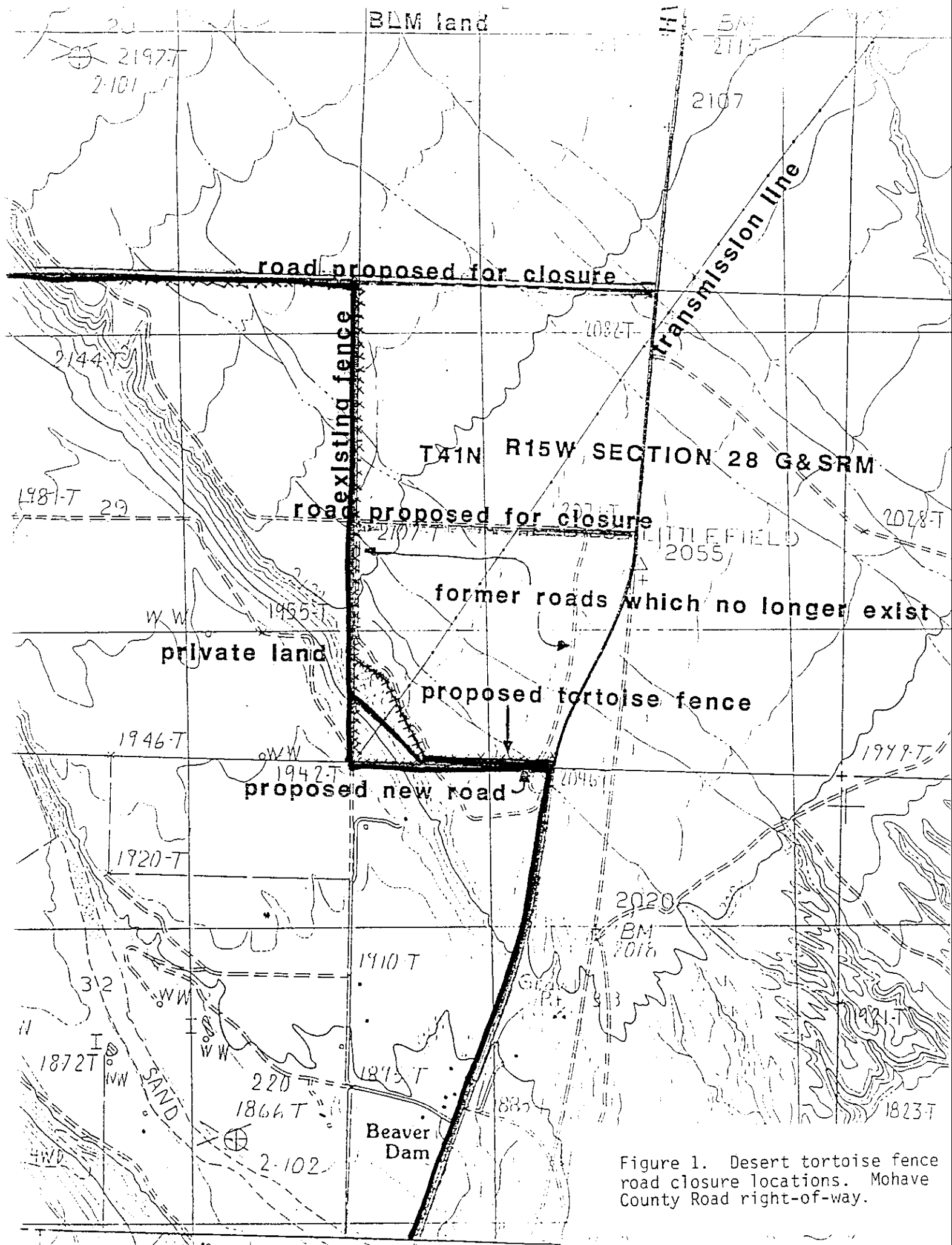
BIOLOGICAL OPINION

It is my Biological Opinion that the proposed action for the issuance of the right-of-way for the Mohave County road is not likely to jeopardize the continued existence of the desert tortoise.

BACKGROUND INFORMATION

Project Description

Mohave County has submitted a request to BLM for approximately 4 acres of land for a right-of-way (A-24577). The right-of-way permit would allow Mohave County to construct a 2,065 foot road to connect Highway 91 to private land in Beaver Dam Wash (Figure 1). The proposed road would provide for improved legal access necessary for fire protection and emergency needs. The proposed access road along the southern boundary of Section 28, T. 41 N., R. 15 W., will run across



flat public land adjacent to the private land from old highway 91 to the brow of the terrace, then turn northwesterly and descend the steep face. The turn and descent is necessary to obtain a six percent grade. Near the bottom, the road will begin a turn to enter private land and the existing roadway. Although not specifically part of this action, it seems certain that an additional 465 feet of roadway will be constructed on private lands in the SE1/4 of Section 29, T. 41 N., R. 15 W.

The County proposes to start construction within 6 months after receiving the right-of-way permit. The right-of-way would be 60 feet wide across the flat land (1380 feet in length) and 84 feet wide on the slope portion (685 foot in length). Construction of the road will not require disturbance of the entire right-of-way. Access to the proposed site will be provided by Highway 91. The site for the road was selected because of its location adjacent to private lands.

The roadway will be built by grading a strip approximately 30 feet wide. The road surface would be capped with recycled asphalt paving to minimize dust. Due to gentle slopes, very little flood control work will be required on the bench. On the steep slope, a cut and fill operation will be necessary, as will some flood control work, requiring the additional 24-foot width. The road would serve approximately 400 to 500 people during the peak habitation season, November to April.

The soils on the proposed site are composed of gravelly fine sandy loams with 2 to 40 percent slopes on a south or west aspect. The primary vegetation consists of creosotebush (Larrea tridentata), white bursage (Ambrosia dumosa), big galleta (Hilaria rigida), Indian ricegrass (Oryzopsis hymenoides), range ratany (Krameria parvifolia), and bladdersage (Salazaria mexicana). Parts of the surveyed acreage show signs of extensive human disturbance as indicated by dirt roads, vehicle tracks, and scattered trash. Since the area is adjacent to developed private lands, there have been some human activities that have adversely modified the area.

The location of the proposed road is adjacent to or near existing private land which will minimize impacts to undisturbed tortoise habitat. The north side of the right-of-way will be fenced to reduce off-road travel into category 1 tortoise habitat and exclude tortoises and livestock from the right-of-way. Two existing roads within the category 1 tortoise habitat will be closed and rehabilitated.

The Rangewide Plan (BLM 1988) objectives for the issuance of rights-of-way state the following:

- (1) Grant new rights-of-way through Category I and II tortoise habitat areas only if no reasonable alternative exists. Mitigation for habitat losses is required.
- (2) Mitigate along rights-of-way to minimize direct losses of tortoises, fragmentation or reduction of habitat, and the effects of construction.

In accordance with the Rangewide Plan, the BLM proposed the following mitigation measures:

1. Measures to reduce dust will be employed such as application of gravel, asphalt or dust suppressants, to the traveled surface (EA #AZ-010-90-022).
2. A tortoise-proof fence to confine traffic to the roadway and exclude tortoises and livestock from the right-of-way will be installed along the flatland and above the face of the terrace and connect with existing fences at highway 91 and Biasi private land (EA #AZ-010-90-022).
3. Two existing roads to the north in Sections 21 and 28, within category 1 desert tortoise habitat, will be rehabilitated and closed. These two roads are one-half mile and one mile north of the proposed road, totalling two miles of roadway (EA #AZ-010-90-022).
4. The right-of-way will be cleared by a qualified biologist prior to construction (EA #AZ-010-90-022).
5. The right-of-way width will be reduced to 60 feet on the benchland portion of the road (EA #AZ-010-90-022).
6. Recommendations resulting from the Section 7 consultation will be followed (EA #AZ-010-90-022).
7. If a tortoise moves onto the site during construction, all activity will cease until the tortoise moves out of harm's way of its own volition (BLM memorandum, September 5, 1990).
8. The qualified desert tortoise biologist will be responsible for informing all foremen, construction workers, and other employees working on this project about the desert tortoise (which will include information provided by the BLM on the life history of the desert tortoise, its protected status, protocols for dealing with tortoises if and when they are encountered, and the definition of take). All workers will also be instructed to check under all vehicles before moving such vehicles (tortoises often take cover under vehicles) (BLM memorandum, September 5, 1990).
9. Lands to be developed within the proposed action site will be fenced with a tortoise-proof fence prior to construction activity and clearances. This fence will remain in place on the north side of the road after construction activity is complete. A tortoise biologist will inspect the fence line prior to construction to ensure that tortoises are not in harm's way (BLM memorandum, September 5, 1990).
11. A tortoise survey will be conducted by a qualified biologist no less than 15 days prior to the surface disturbance activity between the period from March 1 through October 31 and no less than 30 days prior to surface

disturbance activity between the period November 1 through February 28. Surveys will include 100% coverage (transects will be no greater than 10 meters wide) of the proposed surface disturbance sites (BLM memorandum, September 5, 1990).

12. Desert tortoises encountered during pre-construction clearances or during construction will be relocated by the qualified biologist, a minimum of 150 yards into an off-site burrow on public land. If a burrow is not available, one must be constructed by the biologist (BLM memorandum, September 5, 1990).
13. Desert tortoises encountered experiencing heat stress will be placed in a tub with 1/2 inch of water in an environment with a temperature between 76°F and 95°F (BLM memorandum, September 5, 1990).
14. Desert tortoises moved in the winter (i.e. 1 November through 28 February) must be placed into an adequate burrow; if one is not available, one will be constructed, by hand, utilizing the following restrictions (BLM memorandum, September 5, 1990):
 - a. the burrow will be 8 - 10 feet long,
 - b. the burrow will be 2.5 feet deep,
 - c. PVC pipe, of a width approximately equal to the width of the desert tortoise, will be used for the burrow, and
 - d. the temporary burrow should be covered over with the excavated material so that at least 2.5 feet of the back end of the burrow is covered.
15. Garbage and food will be removed daily from the site and will be disposed of in authorized garbage dumps or sanitary landfills (BLM memorandum, September 5, 1990).
16. The Area Manager, BLM, must be notified at (801) 628-4491 when construction of this proposed action begins (BLM memorandum, September 5, 1990).
17. Two other roads within Section 28 between Highway 91 and Beaver Dam Wash are to be closed and rehabilitated (Figure 1). This will include ripping the roadway, removing the gate and wiring the highway right-of-way fence closed (BLM memorandum, September 5, 1990).
18. Activities associated with rehabilitation of these roads will be confined to existing surface disturbance (no new surface disturbance) and shall occur between October 15 and February 15, a period of tortoise inactivity (BLM memorandum, September 5, 1990).

Species Description:

The Beaver Dam Slope population of the desert tortoise, located in southwestern Washington County, Utah, was Federally listed as a threatened species with 39 square miles of critical habitat on August 20, 1980. Subsequently, the Mojave population of the desert tortoise was listed by emergency rule as endangered on August 4, 1989, and by final rule as threatened on April 2, 1990. The Mojave population includes all desert tortoises north and west of the Colorado River in California, southern Nevada, northwestern Arizona, and southwestern Utah, including the Beaver Dam Slope.

The burrowing habits of tortoises, which vary greatly with their geographic locality (Burge 1978, Luckenbach 1982), represent unique adaptations to the extreme environs they occupy. Burrows function primarily as thermo-regulatory aids and may also serve to aid in water conservation and protection from predators. Shelter sites may be located under bushes, in the banks or beds of washes, in rock outcrops, or in caliche caves.

Desert tortoise growth averages 9 millimeters (mm) per year, with the greatest amount of growth following winters of high precipitation and the resultant increase in production of winter annuals in the spring (Medica et al., 1975). Turner et al. (1987) estimated that sexual maturity is attained at an age of 17-20 years. Egg laying occurs from May through July. Nests are dug in sandy soil and usually resemble undisturbed ground. Females often urinate on the nest before and after filling it (Patterson 1971). Clutch size varies from 2 to 14 eggs (5 to 6 being the mean), with larger females generally having larger clutches (Grant 1936, Ernst and Barbour 1972). Forage must be sufficient to allow females to accumulate energy reserves for egg production (Turner et al. 1986). Tortoises are able to increase egg production in good rainfall years by increasing the number of clutches (Turner et al. 1984). The quality and quantity of food available is also important in clutch success (Mayhew, 1968).

Incubation apparently varies from 90 to 120 days in the wild, with hatching occurring from August to October. Observations by Luckenbach (1982) indicate that hatchlings spend little time on the surface, as they either dig or find an existing burrow and begin dormancy shortly after hatching, ignoring food and water. In some cases, eggs do not hatch in autumn but remain over the winter, with hatchlings emerging in the spring.

Peak tortoise activity usually coincides with the abbreviated period of annual bloom in the spring. Luckenbach (1982) considers this spring bloom to be critically important to tortoise survival and reproduction. Tortoises prefer some plants over others and will go out of their way to consume them even when the plant is in low abundance. It is important that tortoises vary their diet because few forage species supply a good balance of nutrients (Urness and McCulloch, 1973).

Average home ranges of tortoises can vary from 11 to 53 hectares (ha), (Berry 1986). Berry also reported extreme long distance movements of 7.2 kilometers (km) over a 15-month period. Additional information on the biology of the desert tortoise can be found in Berry (1984) and Woodbury and Hardy (1948).

The site was surveyed for desert tortoises by a BLM wildlife biologist and an Arizona Game and Fish Department research assistant. Transects (10 meters apart) were walked across the parcel for one hundred percent coverage. The survey included an inspection of an additional area extending 100 meters from the site boundaries.

The survey resulted in the location of one active sheltersite, evidenced by the presence of tortoise scat, along the top of the steep slope. No tortoises or tortoise carcasses were encountered during the survey. No other listed or proposed plant or wildlife species were encountered.

The site has received substantial use and has been adversely affected by past activities. However, the proposed project area is adjacent to relatively undisturbed category 1 tortoise habitat to the north. Tortoise studies have indicated that the highest densities of desert tortoises on the Arizona Strip occur in nearby section 27 (Hohman and Ohmart 1980, Duck and Snider 1988). The proposed road would impact 1.9 acres of Category 1 desert tortoise habitat on the flat land (60 feet x 1380 feet) and 1.32 acres of Category 2 habitat on the steeper slopes (84 feet x 685 feet).

EFFECTS OF THE ACTION

The potential exists for individual tortoises to be killed or injured during construction of the facilities. Construction of the road would also result in permanent loss of 1.9 acres of category 1 habitat and 1.32 acres of category 2 habitat. Construction of the road would destroy one active tortoise sheltersite and impact tortoises whose critical components of home range are within the project area. The new road would also provide improved access to the category 1 desert tortoise habitat to the north. Vehicles leaving the roadway could damage tortoise habitat through crushing of vegetation, soil compaction, disturbance of the soil's crust and reductions in structural variability in desert vegetation (Bury 1980). Additional indirect impacts may occur from noise produced by vehicles (Bury et al. 1977). Impacts due to loss of habitat should be minimized by the reclamation of two miles of roadway within Category 1 tortoise habitat to the north (Figure 1).

Tortoises could also be killed or injured when attempting to cross the road after its completion. Nicholson (1978) found that paved roads and vehicular traffic have a detrimental effect upon tortoise populations within about a mile of a road. The tortoise-proof fence proposed as part of the project should minimize tortoise mortality resulting from increased traffic on the new road.

Construction activity and the associated human presence could also result in an increase in the number of common ravens (Corvus corax) in the vicinity. Ravens are very efficient predators of young tortoises and are attracted to trash generated by human activity. Trash removal programs proposed as part of the project should minimize numbers of ravens due to construction activities.

The FWS does not believe the impacts described above are sufficient to jeopardize the continued existence of the species. We present this conclusion for the following reasons:

1. Two existing roads to the north of the right-of-way will be obliterated and reclaimed. These roads occur within category 1 desert tortoise habitat (Figure 1).
2. Mohave County will implement measures to reduce the take of individual tortoises both during construction and subsequent operation and maintenance of the road. A tortoise-proof fence will prevent tortoises from entering the right-of-way during construction and will reduce mortality due to increased traffic following completion of the road. Tortoises within the right-of-way will be moved to the far side of a tortoise-proof fence to avoid mortality during construction.

Cumulative Effects

Cumulative effects are those effects of future non-Federal (State, local government, or private) activities that are reasonably certain to occur during the course of the Federal activity subject to consultation. Future Federal actions are subject to the consultation requirements established in Section 7 of the Act and, therefore, are not considered cumulative to the proposed action.

The majority of the land north of the right-of-way is Federal land managed by the BLM. Any future activities on these lands will be subject to Section 7 consultation. Certain actions on public lands, such as unauthorized livestock use, off-road vehicle use, and dumping are difficult to control and may contribute to continued habitat loss and degradation. On-going actions on private lands, such as mining, oil and gas leases, sand and gravel operations, grazing, off-road vehicle use, and urbanization may also contribute to continued habitat loss and degradation.

Lands to the south and west of the proposed right-of-way are in private ownership. Some of these lands have already been developed, consisting of single family dwellings, mobile home trailer parks, public service and recreational facilities, roads, schools, and stores. This urbanization impacts tortoise habitat quality and quantity, causes habitat fragmentation, and increases the potential for tortoise mortality due to vehicular traffic and vandalism. Urbanization also results in the increased harassment and mortality to desert tortoises by domestic dogs (Canis familiaris).

Agricultural development has occurred in the area and can be expected to continue. An additional 465 feet of roadway is likely to be constructed on private lands in the SE1/4 of Section 29.

INCIDENTAL TAKE

Section 9 of the Endangered Species Act, as amended, prohibits any taking (harass, harm, pursue, hunt, shoot, wound, kill, trap, capture, collect, or attempt to engage in any such conduct) of listed species without a special exemption. Harm is further defined to include significant habitat modification or degradation that results in death or injury to listed species by significantly impairing behavioral patterns such as breeding, feeding, or sheltering. Under the terms of Section 7(b)(4) and 7(o)(2) of the Act, taking that is incidental to and not intended as part of the agency action is not considered taking within the bounds of the Act, provided that such taking is in compliance with this incidental take statement. The measures described below are nondiscretionary, and must be undertaken by the agency or made a binding condition of any grant or permit issued to the applicant, as appropriate.

Based on the analysis of impacts provided above, the FWS anticipates that the following take could occur as a result of the activities associated with construction of the proposed county road:

1. One tortoise may be taken during construction of the road in the form of harassment through excavation of one active burrow in the construction area.
2. A total of 1.9 acres of category 1 desert tortoise habitat and 1.3 acres of category 2 desert tortoise habitat may be taken for the road right-of-way.

No take should occur in conjunction with operation and maintenance of the road if the proper mitigation measures (i.e. the tortoise-proof fence) are implemented and properly maintained.

The FWS is assuming that the stipulations contained in this Opinion, as Reasonable and Prudent Measures and Terms and Conditions to reduce take, will reduce the potential for take of both individual tortoises and tortoise habitat as a result of the proposed action. These stipulations include measures developed by the BLM, where terms and conditions are not more restrictive. This Opinion does not authorize take in the form of the collection of tortoises for pets. Any person found engaging in such an activity will be liable for prosecution.

Reasonable and Prudent Measures

The FWS believes that the following Reasonable and Prudent Measures are necessary and appropriate to minimize the incidental taking authorized by this Biological Opinion:

1. Habitat loss, degradation, and fragmentation shall be minimized and compensated.
2. Desert tortoise mortality due to construction of the road shall be minimized.
4. Potential for violations of Terms and Conditions shall be minimized.

Terms and Conditions

In order to be exempt from the prohibitions of Section 9 of the Act, the BLM and the project proponent, Mohave County, are responsible for compliance with the following terms and conditions, which implement the reasonable and prudent measures described above. Several of the Terms and Conditions were included in the BLM's Biological Evaluation and Environmental Assessment for this project and are reiterated here for clarity. These Terms and Conditions are as follows:

- 1a. Two existing roads (Figure 1) to the north of the right-of-way in Sections 21 and 28, within category 1 desert tortoise habitat, shall be permanently closed and rehabilitated within 1 year of the issuance of this biological opinion. These two roads are one-half mile and one mile north of the proposed road and total two miles of roadway. The roads shall be ripped to prevent further passage and revegetated with native species to accelerate recovery of the habitat. Access to the roads from the main road shall be permanently blocked. Any vandalism to gain access to the roads shall be corrected within one week of occurrence. Activities associated with rehabilitation of these roads shall be confined to existing surface disturbance (no new surface disturbance) and shall occur between October 15 and February 15, a period of tortoise inactivity.
- 2a. A tortoise survey shall be conducted by a qualified biologist no less than 15 days prior to the surface disturbance activity between the period from March 1 through October 31 and no less than 30 days prior to surface disturbance activity between the period November 1 through February 28. Surveys will include 100 percent coverage (transects will be no greater than 10 meters wide) of the proposed surface disturbance sites. Sheltersites which cannot be avoided shall be excavated by hand during this time period. All excavation of desert tortoise burrows shall be in accordance with the desert tortoise handling procedures (Appendix 1). Measures shall be taken to prevent tortoises from re-occupying the burrow sites. Burrows shall be excavated and tortoises handled only by authorized biologists.

- 2b. All tortoises which are found on the project site, whether above ground or in excavated burrows, shall be placed at a minimum of 150 yards to the north of the right-of-way. Tortoises shall be placed in a burrow on BLM land. If a burrow is not available, one must be constructed by the biologist. Disposable latex gloves shall be used to handle all tortoises. Cardboard boxes used to transport and hold tortoises shall be purchased new, used once, and discarded. All materials which come into contact with tortoises shall be used only once and then properly discarded to minimize contact with the causative factor(s) for Upper Respiratory Disease Syndrome. Tortoises shall be kept upright at all times and handled in a secure but gentle manner to minimize stress, including possible voiding of the bladder.
- 2c. Tortoises shall be purposefully moved only by authorized personnel, solely for the purpose of moving tortoises out of harm's way. If a tortoise is endangered by any form of construction activity, that activity shall cease until a biologist, as described above, is able to move the tortoise to safety.
- 2d. A permanent tortoise-proof fence shall be constructed on the northern edge of the right-of-way and secured to a right-of-way fence (Figure 1). The fence shall be designed to prevent tortoises from entering the right-of-way and to prevent vehicles from accessing the undisturbed desert.
- 2e. The tortoise-proof fence shall be constructed prior to the onset of any other construction activity. This precaution will ensure that tortoises do not enter the project site during construction. The tortoise-proof fence shall also delineate the right-of-way and minimize disturbance of habitat outside of the right-of-way by construction equipment.
- 2f. A qualified tortoise biologist shall be on-site during the construction of the tortoise-proof fence to ensure that construction activities do not harm tortoises. Any tortoises in the path of construction shall be moved in the manner described in Appendix 1.
- 2g. The tortoise-proof fence shall be tied into existing tortoise barriers at the boundaries of the current project or shall be constructed in a manner that would function as a "drift fence" to direct tortoises away from the road or through a culvert under the road.
- 2h. The tortoise-proof fence shall be monitored regularly, including at least twice yearly, and maintained for the life of the project. Monitoring and maintenance shall include, but not be limited to, regular observations to determine whether trash, sediment accumulation, or erosion is compromising the fence's function. Any indications of fence failure shall be reported to the FWS within 30 days of its finding. The tortoise-proof fence shall also be monitored to determine its effectiveness in excluding tortoises from entering the right-of-way.

- 2i. All construction and maintenance vehicles shall stay within the designated right-of-way. Overnight parking and storage of equipment and materials, including stockpiling and borrow sites, shall occur within the designated right-of-way.
- 2j. Construction and maintenance workers shall strictly limit their activities to designated construction areas and routes of travel to eliminate unnecessary adverse impacts to desert tortoise habitat.
- 2k. A litter control program shall be implemented during construction that will include use of covered trash receptacles and prompt removal to an approved disposal site to avoid attracting ravens.
- 3a. Mohave County shall designate an individual as a contact representative who will be responsible for overseeing compliance with protective stipulations for the desert tortoise and coordination with the BLM.
- 3b. All construction and maintenance workers shall be informed of the occurrence of the desert tortoise in the area and its status. They shall be advised as to the potential impacts to tortoises and potential penalties (up to \$25,000 in fines per violation and one year in prison) for taking a threatened species. The contents of the worker and inspector education program shall be submitted to the FWS for review 30 days prior to the presentation of the program.
- 3c. No later than 90 days after the completion of construction within tortoise habitat, the designated biologist (or other appropriate person) shall submit a post-construction biological report to the FWS. This report shall document the effectiveness of the tortoise mitigation measures, the level of take associated with the project, and the status of the road closures.

The FWS is to be notified within three days of the finding of any threatened or endangered species found dead or injured as a result of this action. Notification must include the date, time, and location of the injured tortoise or carcass, and any other pertinent information. Dead animals may be marked in an appropriate manner and left on-site. Injured animals should be transported to a qualified veterinarian. Should any treated tortoises survive, the FWS should be contacted regarding the final disposition of the animals.

Reporting Requirements

Upon locating dead, injured, or sick desert tortoises, initial notification must be made to the FWS' Division of Law Enforcement, Federal Building, Room 8, 26 North McDonald, Mesa, Arizona, (Telephone: 602/261-6443). Instructions for proper handling and disposition of such specimens will be issued by the Division of Law Enforcement. Care must be taken in handling sick or injured animals to

ensure proper treatment and care, and in handling dead specimens to preserve biological material in the best possible state. All tortoise remains shall be frozen immediately and provided to one of the following institutions holding appropriate Federal and State permits per their instructions:

Museum of Vertebrate Zoology, University of California, Berkeley, California
 Los Angeles County Museum of Natural History, Los Angeles, California
 San Bernardino County Museum, San Bernardino, California
 University of Nevada, Department of Biology, Las Vegas, Nevada
 University of New Mexico, Albuquerque, New Mexico

Arrangements shall be made with the institution by the BLM through a biologist prior to construction regarding proper disposition of potential museum specimens. Should none of the above institutions want the tortoise specimens, the remains may be disposed of in any appropriate manner. In conjunction with the care of sick or injured tortoises, or the preservation of biological materials from a dead tortoise, the BLM has the responsibility to ensure that photographs and information relative to the date, time, and location of the tortoise when found, and possible cause of injury or death of each tortoise be recorded and provided to the FWS. Should injured animals be treated by a veterinarian and survive, the FWS should be contacted regarding final disposition of these tortoises.

The BLM will notify the FWS of all tortoises killed, injured, or removed from within the right-of-way within three days of the completion of the construction. The BLM shall submit to the FWS a report on all tortoise-related activities undertaken due to the road construction, including tortoise biologist activities and number of tortoises killed or injured, within 30 days after completion of road construction.

If, during the course of the action, the amount or extent of the incidental take limit is reached, the BLM must immediately reinstitute consultation with the FWS to avoid violation of Section 9 of the Act. Operations must be stopped in the interim period between the initiation and completion of the new consultation if the FWS determines that the impact of additional taking will cause an irreversible and adverse impact on the species, as required by CFR 402.14(i). The BLM should provide an explanation of the causes of the taking.

CONSERVATION RECOMMENDATIONS

Section 7(a)(1) of the Act directs Federal agencies to utilize their authorities to further the purposes of the Act by implementing conservation programs for the benefit of endangered and threatened species. Conservation recommendations have been defined as FWS suggestions regarding discretionary agency activities to minimize or avoid adverse effects of a proposed action on listed species or critical habitat, or regarding development of information. The recommendations provided here relate only to the proposed action and do not necessarily represent complete fulfillment of the agency's Section 7(a)(1) responsibility for these species.

1. The BLM should compile guidelines of desert reclamation techniques that can be used as a standard package to be included in all restoration efforts.
2. The BLM should assess the impacts that existing roads on public lands have on desert tortoises.
3. The BLM should consider closing other roads within high density desert tortoise habitat to reduce human-induced impacts.
4. The BLM should revegetate disturbed areas associated with construction of the road with native species.
5. The BLM should ensure that construction and maintenance vehicles do not exceed a speed of 25 m.p.h. in desert tortoise habitat.
6. The BLM should prohibit the discharge of firearms on all construction and maintenance sites in desert tortoise habitat.
7. The BLM should provide increased numbers of rangers to patrol public lands on the Arizona Strip District to enforce BLM restrictions on dumping of trash, unauthorized off-road vehicle use, unauthorized disposal of hazardous waste materials, and other ongoing problems that conflict with protection of the tortoise and its habitat.
8. Because a significant percentage of the habitat occupied by the desert tortoise occurs on BLM lands, the BLM should prepare a cumulative effects analysis of its actions on the desert tortoise throughout its range. This program could include development of a model to assess the effects of past, ongoing, and future projects on the tortoise and its habitat through the use of the Geographical Information System (GIS).

In order for the FWS to be kept informed of actions that either minimize or avoid adverse effects, or that benefit listed species or their habitat, the FWS requests notification of the implementation of any conservation recommendations.

CONCLUSION

This concludes formal consultation on Mohave County right-of-way. As required by 50 CFR 402.16, reinitiation of formal consultation is required if: 1) the amount or extent of incidental take is reached, 2) new information reveals effects of the agency action that may impact listed species or critical habitat in a manner or to an extent not considered in this opinion, 3) the agency action is subsequently modified in a manner that causes an effect to a listed species or critical habitat that was not considered in this opinion, and 4) a new species is listed or critical habitat designated that may be affected by the action.

The FWS appreciates the assistance and cooperation of your staff throughout this consultation process. If we may be of further assistance, please contact Sherry Barrett or me (Telephone: 602/379-4720 or FTS 261-4720).

A handwritten signature in cursive script, reading "Sam F. Spiller".

Sam F. Spiller

Attachment

cc: Field Supervisor, Fish and Wildlife Service, Reno, Nevada
Field Supervisor, Fish and Wildlife Service, Salt Lake City, Utah
Field Supervisor, Fish and Wildlife Service, Ventura, California
Office Supervisor, Fish and Wildlife Service, Laguna Niguel, California
State Director, Bureau of Land Management, Phoenix, Arizona
Director, Arizona Game and Fish Department, Phoenix, Arizona
Regional Director, Fish and Wildlife Service, Albuquerque, New Mexico
(FWS/HC)
Director, Fish and Wildlife Service, Washington, DC (EHC)

LITERATURE CITED

- Berry, K. H. (ed.). 1984. The status of the desert tortoise (Gopherus agassizii) in the United States. Unpubl. Rept. to Fish and Wildlife Service from the Desert Tortoise Council. Cont. No. 11310-0083-81.
- Berry, K. H. 1986. Desert Tortoise (Gopherus agassizii) relocation: implications of social behavior and movements. *Herpetologica* 42(10:113-125).
- Burge, B. L. 1978. Physical characteristics and patterns of utilization of cover sites by Gopherus agassizii in southern Nevada. Proc. Symp., The Desert Tortoise Council. 1978:80-111.
- Bury, R. B. 1980. What we know and do not know about off-road vehicle impacts on wildlife. In: R.N. Andrews and P. F. Nowak (Eds.) Off-road vehicle use: A management challenge. Office of Environmental Quality, U. S. Department of Agriculture and University of Michigan, School of Natural Resources and Extension Service 348 pp.
- Bury, R. B., R. A. Luckenbach, and S. D. Busack. 1977. Effects of off-road vehicles on vertebrates in the California Desert. USDI Fish and Wildlife Service, Wildlife Research Report 8. Washington D.C.
- Duck, T.A. and J.R. Snider. 1988. Analysis of a desert tortoise population and habitat on the Beaver Dam Slope, Arizona. Bureau of Land Management, Arizona Strip District.
- Ernst, C. H. and R. W. Barbour. 1972. Turtles of the United States. University of Kentucky Press, Lexington, Kentucky. 347 pp.
- Grant, C. 1936. The southwestern desert tortoise Gopherus agassizii. *Zoologica*. 21:225-229.
- Hohman, J. and R. D. Ohmart. 1980. Ecology of the desert tortoise (Gopherus agassizii) on the Beaver Dam Slope, Arizona. Unpubl. Rept. submitted to the Bureau of Land Management, Arizona Strip District, St. George, Utah. Cont. No. YA-510-PH7-54.
- Luckenbach, R. A. 1982. Ecology and management of the desert tortoise (Gopherus agassizii) in California. Pp. 1-37 In: North American Tortoises: Conservation and Ecology. R. B. Bury (Ed.). U. S. Fish and Wildlife Serv. Wildlife Res. Rept. 12.
- Mayhew, W. W. 1968. Biology of desert amphibians and reptiles, Chpt. 6. In: G. W. Brown, Jr., (ed.), Desert Biology, Vol. 1. 1968. Academic Press, New York.

- Medica, P. A., R. B. Bury, and F. B. Turner. 1975. Growth of the desert tortoise (Gopherus agassizi) in Nevada. *Copeia* 1975:639-643.
- Nicholson, L. 1978. The effects of roads on desert tortoise populations. In M. Trotter (Ed.) Proc. 1978 Desert Tortoise Symposium, Las Vegas, Nevada.
- Patterson, R. 1971. The role of urination in egg predator defense in the desert tortoise (Gopherus agassizi) *Herpetologica* 27:197-199.
- Turner, F. B., P. Hayden, B. L. Burge, and J. B. Roberson. 1986. Egg production by the desert tortoise (Gopherus agassizii) in California. *Herpetologica* 42(1):93-104
- Turner, F. B., P. A. Medica, and R. B. Bury. 1987. Age-size relationships of desert tortoise (gopherus agassizi) in Southern Nevada. *Copeia* 1987(4):974-979.
- Turner, F. B., P. A. Medica, and C. L. Lyons. 1984. Reproduction and survival of the desert tortoise (Scaptochelys agassizii) in Ivanpah Valley, California. *Copeia* 1984(4):811-820.
- Urness, P. J. and C. Y. McCulloch. 1973. Deer Nutrition in Arizona chaparral and desert habitats. Part II: Chemical analysis and in vitro digestibility of seasonal deer forages. Special report No. 3. Prepared and published under the provisions of the Federal Aid in Wildlife Restoration Act, Project W-78-R, September 1973.
- U.S. Department of the Interior, Bureau of Land Management. 1988. Desert tortoise habitat management on the public lands: A rangewide plan. Bureau of Land Management, Division of Wildlife and Fisheries, Washington, D.C. 23 pp.
- U.S. Department of the Interior, Bureau of Land Management. 1990. Section 7 consultation (desert tortoise) on a right-of-way for a road for Mohave County. Memorandum dated September 5, 1990, to Field Supervisor, Fish and Wildlife Service, Phoenix, Arizona.
- U.S. Department of the Interior, Bureau of Land Management. 1990. Draft Environmental Assessment #AZ-010-90-022, Mohave County/Beaver Dam Road Right-of-Way, A-24577. Transmitted by letter dated August 14, 1990, to Field Supervisor, Fish and Wildlife Service, Phoenix, Arizona.
- U. S. Department of the Interior, Bureau of Land Management. 1990. Additional information for Section 7 consultation on Mohave County Road Right-of-way. Memorandum dated November 21, 1990, to Field Supervisor, Fish and Wildlife Service, Phoenix, Arizona.
- Woodbury, A. M. and R. Hardy. 1948. Studies of the desert tortoise, Gopherus agassizii. *Ecol. Monogr.* 18:145-200.

INTERIM TECHNIQUES HANDBOOK
FOR COLLECTING AND ANALYZING DATA ON
DESERT TORTOISE POPULATIONS AND HABITATS

JUNE 1990

ARIZONA GAME AND FISH DEPARTMENT
CALIFORNIA STATE RESOURCE AGENCIES
NEVADA DEPARTMENT OF WILDLIFE
UTAH DIVISION OF WILDLIFE RESOURCES
UNITED STATES DEPARTMENT OF THE INTERIOR
BUREAU OF LAND MANAGEMENT
FISH AND WILDLIFE SERVICE

III. PROTOCOLS FOR HANDLING LIVE TORTOISES

TABLE OF CONTENTS

Disease Precautions.	III-1
General Handling Protocol.	III-2
Health of Live Tortoises	III-6
Blood Collecting Procedures.	III-6
Suggested Products for Blood Collection and Processing . .	III-8
Protocol for Mycoplasma and Aerobe Culture	III-10
List of Materials for Microbial Culture.	III-11
Marking and Identifying Live Tortoises	III-11
Literature Cited	III-15

III. PROTOCOLS FOR HANDLING LIVE TORTOISES

Field workers should always utilize the least stressful methods when handling tortoises for weighing, measuring or taking tissue and other samples.

Disease Precautions

1. Because of the threat of Upper Respiratory Disease Syndrome (URDS), all tortoises will be handled so as to minimize the chances of spreading disease, even if URDS has not been documented in a given locality. All personnel handling tortoises should wear disposable gloves (latex or plastic are preferred) to prevent transmission of diseases among tortoises. No more than one tortoise should be handled with each pair of gloves. Dispose properly of possibly contaminated gloves, newspapers, cardboard boxes, etc.

2. If more than one tortoise is being held at the same time for measuring or sampling, provide a separate, disposable container for each tortoise. New cardboard boxes are recommended. Bundles of new boxes do not take up much space and the boxes can be broken down easily for disposal.

3. Equipment that comes in contact with a tortoise should be sterilized before it is used on another tortoise. Triangular files used for marking marginal scutes, the tips of calipers used to measure tortoises, rules and other similar equipment should be sterilized by soaking in 95% isopropyl or ethyl alcohol for at least 20 minutes before using on another tortoise. (A 25% solution of chlorine bleach may also be used. Be warned that the bleach is extremely corrosive and may damage many types of equipment). Wooden rules should not be used; they are more difficult to sterilize than metal or plastic ones because of the porosity of the wood and they tend to warp under the prescribed sterilization.

4. To avoid having to sterilize spring scales or weighing straps prior to weighing each tortoise, use individual "T-shirt bags", the plastic bags with the two straps that are used to bag groceries, to suspend the tortoise while weighing. A new bag should be used for each tortoise. These bags may be purchased from grocery supply or cooperative discount houses in quantity for only a few cents apiece.

5. Clothes should be changed completely, including shoes, before visiting other tortoise sites. (As a general rule, a single valley or desert mountain range would be considered one site, unless there were special circumstances, such as URDS confirmed in one part of a valley, but not thought to occur in other parts of that valley. In such an instance, a change of clothes would be

6/30/90

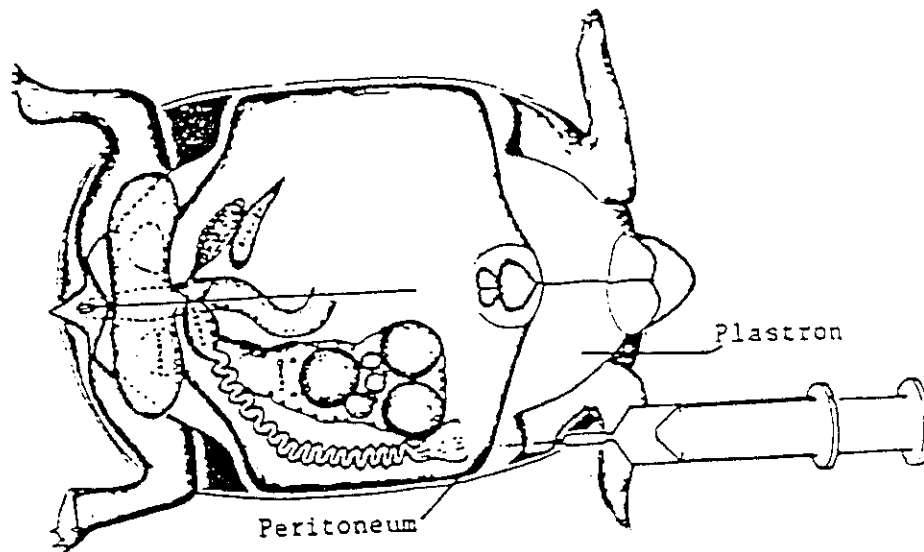


Figure III-1. Ventral view of desert tortoise showing proper placement of needle for rehydration. The distal portion of the plastron has been cut away to show the relationship of internal structures. THE TORTOISE SHOULD REMAIN IN AN UPRIGHT POSITION FOR THIS PROCEDURE. THE VENTRAL VIEW PRESENTED IS ONLY TO ILLUSTRATE PROPER NEEDLE PLACEMENT. Modified from Woodbury and Hardy (1948).

complexed-iodine scrub solution (Betadine or Medadine, for example). The re-hydrating solution (Normosol and dextrose/saline mixture) should be injected using a 1 inch, 22 gauge needle. Insert the needle as shown in Fig. III-1. THE TORTOISE SHOULD REMAIN UPRIGHT. FIG. III-1 IS SHOWN IN VENTRAL VIEW ONLY TO SHOW THE PLACEMENT OF THE NEEDLE FOR REHYDRATION.

Before injecting any fluid, pull the plunger of the syringe slightly backwards to create a vacuum. If blood or bile (a dark, greenish fluid) appears in the syringe, stop aspirating, withdraw the needle slightly and aspirate again to assure that the needle is not in a blood vessel or lobe of the liver.

If more than one syringe of fluid is to be injected, do not remove the needle between injections. Simply remove the empty syringe while holding the needle in place and attach the full syringe, ensuring that no air bubbles are introduced. Discard the needle safely. Do not use a needle on more than one tortoise or use a non-sterile needle to draw fluids.

The needle should enter the skin below and behind the left shoulder joint at its most lateral extent. The needle should parallel the plastron, with the fluid injected just behind the attachment of the pectoral musculature to the plastron and between the plastron and the peritoneum (Fig. III-1).

6/30/90

Normosol-R is produced by Abbott Laboratories (800-222-6883) and can be purchased from veterinary or medical supply houses in most larger cities. The 2.5% dextrose in half-strength saline can also usually be purchased from local veterinary or medical supply houses. (Sometimes laboratories will dilute 5% dextrose in normal saline with distilled water to achieve the desired concentrations). Syringes, needles and other supplies can be purchased from any scientific, medical or veterinary supply house. See suggested lists of suppliers later in this chapter.

The 1:1 mixture will assure a slightly hypotonic replacement, and should maintain normal ionic balance. This is especially important during periods of drouth, such as experienced in many desert tortoise localities the past few years. For 1991, consideration is being given to hydrating apparently desiccated tortoises or those from areas of severe drouth, whether they void or not.

3. To prevent hyperthermia, on warm days a tortoise must be kept in the shade (of the fieldworker, a pack, other equipment etc.) except during photography. Tortoises should not be processed (weighed, measured, etc.) when air temperatures exceed 90 degrees F (32 C) at 1.5 m (4.9 ft) above ground, unless measures are taken to insure the animal does not overheat. If tortoises are collected early in the day when it is cooler and held for processing during midday, take care to provide sufficient shade and cooling for the tortoises while holding and processing them. Insure that the animals always have sufficient shade, especially as the sun moves across the sky during the day. CAUTION! TEMPERATURES ARE MUCH HIGHER NEARER THE GROUND! Take extreme caution to avoid overheating of tortoises whenever surface temperatures exceed 86 F (30 C). Shield the bulb of the thermometer from direct solar radiation when measuring temperatures.

4. Carelessly turning a tortoise on its back or spinning it can cause serious problems such as pulmonary edema, internal egg breakage, psychogenic shock and possibly intestinal torsion. If a tortoise must be turned over on its back, it should not be spun around or repeatedly turned over. The fieldworker should return the tortoise to the upright position by carefully rolling it back over in the same direction. The tortoise should not be rolled end over end, side over side, or spun.

5. Tortoises, especially females, may be fatally damaged by blows, butting, or overturning, which results in egg yolk peritonitis brought on by seepage of egg yolk or breakage of shelled eggs into the peritoneal cavity (Rosskopf and Woerpel 1982, Yanoff and Rosskopf 1983). Egg yolk can cause a serious inflammatory reaction. Handling of potentially gravid females should be done very carefully. An aggressive, courting male can easily kill an egg-carrying female. Such instances may occur mostly in captivity, where females cannot easily escape the advances of the male. A careless fieldworker could also cause breakage of eggs,

6/30/90

especially if they are thin-shelled. Rosskopf and Woerpel (1982) state:

Clients should be advised to carefully observe all female tortoises of egg-laying age and to avoid trauma to them, especially any activity that may lead to the tortoise turning over on its back. Tortoises with uric acid cystic calculi are especially prone to this condition, due to the trauma-inducing nature of these foreign bodies on the neighboring ovarian tissue.

6. Remove a tortoise from its cover site only if the cover site will not be damaged in the process. Pallets and burrows can be damaged or destroyed in attempts to capture tortoises. Pallets and burrows of juvenile tortoises are particularly vulnerable. If the tortoise is out of reach in the cover site, do not break away the opening or otherwise damage the burrow to reach the tortoise. Instead, flag the site and return later in the day or early the next day in hopes of capturing the individual when it emerges from the cover site or is closer to the entrance.

Often tortoises within reach in the burrow are difficult to remove, because they will firmly press the shell against the roof and tense the limbs against the burrow floor. In tugging and pulling to remove the tortoise, parts of the burrow can break away. One way of avoiding damage is to gently remove soil from the floor of the burrow and from beneath the tortoise with the hand, thereby increasing the height of the burrow. The tortoise is then more easily pulled out and the soil can be replaced.

Tim Shields, Karen Bohuski, and other fieldworkers have expressed concern about removing juveniles from their cover sites. They suggest that juveniles are particularly sensitive to handling, and once pulled from a cover site may leave the site and not return in the short term, if ever. Instead, these fieldworkers recommend that juveniles be captured when outside the cover site. They use flagging and frequent visits to the site to capture the juvenile and are often successful. Juveniles captured outside the cover sites apparently show no reluctance to return to the cover site.

7. When transporting a tortoise, insure that the tortoise is as secure as possible so that it will not slide around in its transport box while being driven from or back to the field. Minimize the stress of transport as much as possible by minimizing temperature differentials between its environment and the holding box or container.

8. The tortoise should be returned to the place where it was found (preferably it would not be moved at all) and should not be taken some distance away for release. Cases have arisen where

6/30/90

fieldworkers have conducted personal, unauthorized experiments, and have moved resident tortoises from one place to another to "see what would happen." Such practices are not authorized and could result in the revocation of one's Federal collecting permit.

The above are general rules. If the shells of the tortoises are abnormally thin, soft, or lack proper ossification, such as some of the tortoises from the Beaver Dam Slope of Utah and Arizona, then special measures should be instituted during handling to prevent damage to the tortoises.

Health of Live Tortoises

Fieldworkers should continue to record information on anomalies, injuries, signs of disease and parasites. What is the condition of eyes and nose? Is there dried mucous on the face or front limbs? Is the tortoise wheezing? What is the condition of the shell, including chew and bite marks (old and recent) on the shell and limbs? Note the presence and location of replacement bone; presence and location of sunken scutes; and shell wear class. If problems or symptoms of disease exist with the animal, slides should be taken to document the problem, in addition to extensive notes on the data sheet.

Blood Collecting Procedures

There is a need to standardize blood sampling procedures so that results from different physiological studies can be compared. In this section we are referring to blood samples ≥ 0.5 ml. The three principal methods of taking blood that are currently being used or are proposed for use are brachial and jugular venipuncture and cardiac puncture. Jugular venipuncture is the recommended method. However, because some studies are in progress using brachial venipuncture, that technique may also be used in those studies until results of tests comparing the techniques have been analyzed.

Cardiac puncture is not recommended for sampling wild desert tortoise populations at this time. Cardiac puncture may be appropriate for certain laboratory experiments and should be evaluated on a case-by-case basis.

Jugular venipuncture

Three individuals are needed per tortoise for jugular venipuncture: one for manual restraint/neck extension and venipuncture, one to draw the blood into the syringe, and one to handle the sample tubes, determine packed cell volumes, and prepare coverslip films or smears.

6/30/90

1. Extend the neck of the tortoise and visualize the jugular vein. (If a blood sample cannot be obtained from the jugular vein, a sample should be obtained from the brachial vein). Clean the area of skin over the vein with 70% ethanol. Insert a 23-gauge butterfly infusion catheter into the vein. Collect 6cc (for a tortoise >1000 grams) of blood in a 10cc syringe. Hand the syringe quickly to the third person who should be waiting with appropriate tubes. After the catheter needle is withdrawn, the person restraining the tortoise should apply gentle pressure to the puncture site to prevent bleeding and hematoma.

The likelihood of blood clotting during analysis may be decreased by coating the inside of the butterfly catheter with lithium heparin prior to the blood collection. Add 4cc of saline to a 5cc lithium heparin tube. Draw up a small amount of the saline and lithium heparin solution and flush it through the catheter so that only a small film remains on the catheter.

2. Place approximately 0.6cc of blood into a lithium microtainer tube for complete blood count (CBC) and hemoglobin determination. Place the remainder of the blood into a 5cc lithium heparin tube. Cap the tubes quickly and invert several times to ensure that the anticoagulant is properly mixed with the blood. Place the tube on the hematology mixer as soon as possible. The hematology mixer is recommended if many blood samples are to be taken. If only a few samples are being taken for other studies, take care to gently mix the samples. After mixing for a few minutes, place the microtainer tube in a box on wet ice or in a cooler with ice packs. DO NOT FREEZE THIS SAMPLE. Ship the samples by Federal Express or similar carrier the same day.

3. Draw a small quantity of blood from a lithium heparin tube up into a microhematocrit tube for packed cell volume (PCV) determinations. Seal the end of the tube properly before centrifugation. Use a small drop of blood for preparing blood films. Identify microscopic slides using a Fisher brand Histoprep Film Secure-Line Marker. Fix the microscopic slide blood films in absolute methanol, store them in a slide box, and ship along with the blood.

4. Centrifuge the remaining 5cc lithium heparin tube within 45 minutes and transfer the plasma to "O-ring" Sarstedt tubes. Place the Sarstedt tubes in liquid nitrogen. Try to standardize the period the blood samples are held before centrifuging. Remove the plasma as soon as possible or hold on ice while waiting to spin it down. At room (or field) temperature, the blood parameters can change while waiting to be centrifuged (potassium leaks out of the red blood cells, for instance). The receptacles in the centrifuge that hold the tubes should be kept on ice until the sample is centrifuged so as not to warm the blood.

6/30/90

Make sure that the tubes are properly marked with a Sharpie brand, permanent marking pen. Use P as a prefix indicating plasma.

Check the liquid nitrogen container daily and refill as needed (probably every 4 to 6 weeks). The plasma samples in the "O-ring" Sarstedt tubes may be sent to the processing laboratory on dry ice in a well-insulated container once all samples have been collected for a particular sampling period.

Suggested Products for Blood Collection and Processing

The following list of products are suggested to facilitate the work of personnel unfamiliar with medical and veterinarian supplies. Of course, other suppliers may be used, especially if smaller quantities of materials are needed. Quantities needed will vary with the project.

Item	Company	Catalog No.	Quantity
1. TRIAC combination centrifuge	Fisher	05-100-80	1 each
2. Syringes			
3cc	Fisher	14-823-39	1 case
10cc	Fisher	14-823-140	1 case
3. Needles			
23 gauge 1"	Fisher	14-826A	5 pkg
22 gauge 1"	Fisher	14-826B	5 pkg
4. Capillary tubes	Fisher	02-668-66	2 pkg
5. Hemat-O-Seal	Fisher	02-678	1 pkg
6. Lithium heparin microtainer tubes	Fisher	02-668-75	1 case
7. Lithium heparin tubes -- 5cc	Fisher	02-687-81	1 case
8. Coverslips 22mm squares	Fisher	12-540-100	1 pkg

6/30/90

Item	Company	Catalog No.	Quantity
9. Histoprep Film Secure Line Marker II	Fisher	14-905-30	1 pkg
10. Hematology/chemistry mixer	Fisher	14-059-346	1 each
11. Pediatric tube adap- ters (for 10 above)	Fisher	14-059-345	1 each
12. Plastic transfer pipettes	Fisher	13-771-7	1 pkg
13. Microscope slides	Fisher	12-550-13	1 gross
14. Slide storage box	Fisher	03-450-5	10 each
15. Coplin staining jar- polyethylene	Fisher	08-815-10	6 each
16. Liquid nitrogen tank (LNT) SC 20/20	Specialty Gases S.E.		1 each
17. Canes (for LNT)	Specialty Gases S.E.		30 each
18. Sarstedt tubes 2cc O-ring serum storage tubes	Sarstedt	72.694.006	1000 each
19. Polycarbonate special screw-cap micro-tube racks	Sarstedt	93.1428	1 each
20. Styrofoam storage boxes	Sarstedt	95.064.249	20 each
21. Absolute methanol	Purchase locally		
22. 70% ethanol	Purchase locally		

Telephone Numbers:	Abbott	- 800-222-6883
	Fisher	- 714-669-4600
	Sarstedt	- 800-321-5680
	Specialty Gases S.E.	- 800-344-1872

6/30/90

Protocol for Mycoplasma and Aerobe Culture

In general, at least two persons are required to obtain culture specimens, one to stabilize the tortoise in the proper orientation and the other to obtain the specimens. Since the tortoise often finds these procedures annoying, it is important that the tortoise be well-controlled so that procedures do not become unnecessarily lengthy and specimen quality is maintained.

1. Cleansing the external nares.

While the tortoise is stabilized, thoroughly moisten a sterile polyester swab with one tube of sterile saline. Use this saline-soaked swab to gently superficially cleanse the area around both external nares. Discard the swab. Repeat the procedure with a fresh saline-soaked swab.

2. Collecting the broth/flush specimen.

Use sterile technique to draw up about 2 cc of a tube of trypticase soy broth (TSB) into a sterile 3cc syringe to which a tomcat catheter has been affixed. Judge the size of the tortoise's nares by visual inspection and, with a flame-sterilized scissors (cooled), cut the catheter so that it will fit in a naris. The catheter will now be 2 to 3 cm long (1 to 1.5 inches) and will have an elliptical opening which will fit into the naris. Eject excess air from the syringe. Insert the tip of the catheter into one naris about 5mm, depending on the size of the tortoise. It is important that the catheter not be inadvertently contaminated by touching any part of the tortoise except the naris being sampled. If such contamination does occur, immediately discard the contaminated catheter and attach a fresh sterile catheter to the syringe. Inject about 1cc of TSB into the naris and pull back immediately on the plunger to aspirate broth and other material back into the syringe. This injection-withdrawal motion may be repeated. Place approximately 1cc of the aspirated broth into a 2cc Sarstedt tube. A separate syringe should be close at hand in order to perform the same procedure on the opposite naris; the same tomcat catheter can be used. Perform the same procedure on the opposite naris and add 1cc of the aspirate to the Sarstedt tube containing the aspirate from the first naris.

3. Handling the samples.

Label all Sarstedt tubes properly, utilizing the prefix M for microbial. Immediately place all tubes into liquid nitrogen.

6/30/90

List of Materials for Microbial Culture

Item	Company	Catalog Number	Quantity/ Unit
1. Saline, 5cc tube	Remel	08-756	72/cs
2. Dacron-tipped swab	Remel	55-025	100/pg
3. 3.5 Fr. Tom Cat Catheter	Sherwood Medical	HRI 8890-703021	50/cs
4. Trypticase Soy Broth	Fisher	BB21404	100/cs

Telephone Numbers:

Fisher	- 714-669-4600
Remel	- 800-255-6730
Sherwood Medical	- 805-526-9071

Marking and Identifying Live Tortoises

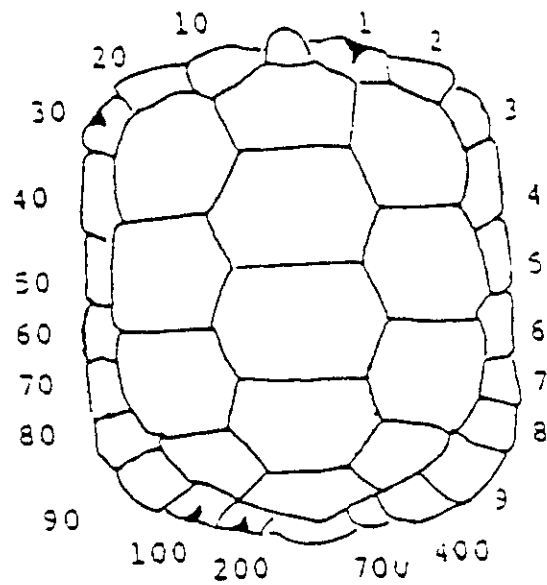
Tortoises and turtles have been identified using one or more of several semi-permanent techniques, such as branding (Woodbury and Hardy 1948), drilling of holes in anterior and posterior marginals, and notching with a three-cornered file or saw. Desert tortoises on BLM study plots have been marked with two notching systems, one of which has been used on the majority of the plots (Fig. III-2). If a particular numbering system has been used on a plot, it should not be changed to another without compelling reasons.

Make notches with triangular files of various sizes on all sizes of tortoises, from juveniles of about 36 mm in length to large adults of 380 mm carapace length. Larger tortoises require larger files. Finger or toenail clippers or small scissors can be used on hatchlings and small juveniles to cut through the shell.

Notch depths will vary with the size of the tortoise and location on the carapace. Depth may range from 2 to 3 mm on a hatchling to 10 to 12 mm on a tortoise with flared marginals. If the tortoise is very small, select an identification number (out of sequence, if necessary) that allows notching of anterior and posterior marginals only, not the bridge. Notches should be deeper on anterior and posterior marginals than on the bridge, because these scutes are more vulnerable to wear and chewing by predators and because the bone is farther from the marginal edge, especially in young animals.

6/30/90

Tortoise number = 331



Tortoise number = 331

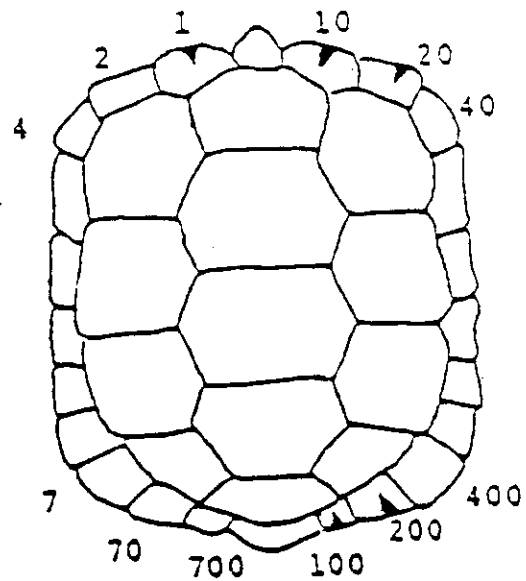


Figure III-2. Coding system (notching) for desert tortoises. Top: System used on most BLM plots in California, Nevada and Arizona. (Some workers do not use the bridge scutes [marginals four through seven] when marking turtles or tortoises). Bottom: System used on BLM plots in Utah and on the Arizona Strip.

6/30/90

Several researchers do not mark the bridge scutes (marginals four through seven) on turtles because of the difficulty in identifying the shallow notches years later (J. Congdon, W. Gibbons and P. Rosen fide C. Schwalbe). This may be particularly troublesome in more complex tortoise habitats in the Sonoran Desert, where a given tortoise may not be recaptured for several years. Arizona is considering this issue and may require that bridge scutes not be used there in the future.

Take care to avoid deep cuts that could cause bleeding. Generally bleeding does not occur unless notches pass through bony tissue. If notches are too deep and bone is damaged, regeneration may occur and the area of damage will be sloughed. For example, bone and scute replacement is now occurring on several tortoises at the Ward Valley plot. Several tortoises first notched in 1980 and recaptured in 1987 have barely recognizable notches. The only evidence of the old notches is a thin fragment of lifting bone distal to scute material.

To avoid notching too deeply on anterior and posterior marginals, tilt the file at an angle to create a groove on the dorsal surface of the scute (Burge fide K. Berry). The groove may be 3 to 5 mm long. On the bridge, angle the file either anteriorly or posteriorly to create a diagonal groove 12 to 20 mm in length. The notch will be less likely to be mistaken for a natural groove, injury or anomaly.

With a wire (strip the paper from a commercial twist-tie), line the notches carefully with yellow ochre acrylic paint, making them slightly more visible to the observer and less likely to be overlooked, if the tortoise is not recaptured and renotched frequently. When a tortoise is recaptured for the first time after a year or more, the notches should be carefully examined and deepened where necessary. The yellow ochre was chosen to match the color of lichens frequently found in the Southwest on rocks.

When identifying a tortoise and when first marking it, the fieldworker should carefully count the marginal scutes from anterior to posterior. This is very important. Many tortoises have 10 or 12, instead of the normal 11 marginal scutes on each side. Other anomalies are common. If the tortoise only has other than 10 marginal scutes, then it should be assigned a number suitable to its scute configuration. When recapturing a tortoise, fieldworkers often see notches on the scutes adjacent to the pygal and assume that the tortoise is in the 200 or 700 series of numbers, when it is not. Always count the marginals from the anterior before identifying the tortoise.

Consideration should be given to techniques for marking juveniles. Clipping with fingernail scissors may be less traumatic than notching. Identification might also be assured with tiny,

6/30/90

epoxy-covered numbers on the costal scutes. If epoxy is to be used, it should not touch the seams of the scutes.

In addition to notching, three other methods should be used concurrently:

- placing a tiny number on the right 4th costal and covering it with quick-drying epoxy, and
- taking a 35 mm photograph (slide) of the carapace, the plastron, and the 4th left costal scute.
- drawing any anomalies, injuries (old and recent), or abnormalities on the Live Tortoise Data Sheet.

In 1977 Betty Burge began testing a new identification system with wild tortoises on the permanent plots in Ivanpah Valley and at Goffs. She placed a tiny piece of paper with the number of the tortoise on the 4th costal scute and covered the number and paper with a drop of quick-drying epoxy. Those numbers have persisted for 10 years, and the system or a modification of it has been adopted on all California plots. It is a valuable supplementary form of identification. The revised system now involves placing a small dot of white paint on the 4th left costal, writing the tortoise number in permanent black ink on the white paint after it is dry, and then covering the number with epoxy. The epoxy will become scratched and the number obscured with time, but when a drop of water is rubbed on the dulled epoxy, the observer can read the number. If properly done, the dot of paint and epoxy will not be obvious to most observers. Dust will cover it. Do not let the epoxy touch the seam.

The 35-mm slides are another essential part of the identification system and have permitted identification of tortoises which have been misidentified, have lost notches, have been chewed extensively by predators, and have died. Even when only a few scutes or pieces of bone remain, the tortoise can sometimes be identified with the slides. If slides are carefully taken, they can show anomalies, injuries, shell wear, and the notches. Slides are preferable to prints, because they are more easily labelled and stored, and can be projected on a screen to compare year-to-year differences.

Before taking the photographs, dust off the tortoise with a small brush (tooth brush), which should be sterilized between tortoises as described above. Many tortoises are caked in mud, and it is difficult, if not impossible, to see seams, shell wear class, anomalies, and growth rings if the shell is not clean. A small piece of paper with the study site number, site name, date, and tortoise number should be affixed to the shell edge (not the center of the carapace or plastron). Plain white stickers 16 mm x 90 mm, used for identifying file folders, are particularly easy to use and

6/30/90

store. The tortoise, carapace, plastron or individual scute being photographed should fill the entire slide frame.

LITERATURE CITED

Rosskopf, W. J., Jr., and R. W. Woerpel. 1982. Egg yolk peritonitis in a California desert tortoise. California Veterinarian 3:13-15.

Woodbury, A. M., and R. Hardy. 1948. Studies of the desert tortoise, *Gopherus agassizi*. Ecol. Monogr. 18:145-200.

Yanoff, S. R., and W. J. Rosskopf. 1983. Mycotic septicemia in a California desert tortoise. Chelonian Documentation Center Newsletter 2:4.

6/30/90